

## Hydrogen peroxide

**CAS ID:** 7722-84-1

**Chemical formula:** H<sub>2</sub>O<sub>2</sub>

**Synonyms / Trade names:** Peroxide, hydrogen dioxide, Perox-Aid®

**Chemical composition:** Hydrogen peroxide is the simplest peroxide, which are compounds with a single bond between two oxygen atoms. It is a liquid at room temperature, with a melting point of approximately 0.43° C, and decomposes between 150 – 152° C. Hydrogen peroxide is slightly denser (density of 1.44 g/cm<sup>3</sup>) and more viscous than water. Concentrated solutions appear light blue in color. Its molecular weight is 34.015. Commercial hydrogen peroxide solutions used at fish hatcheries contain 35% hydrogen peroxide, with the remainder being water. The 35% solution is then diluted to the desired exposure concentration.

**Hatchery use:** Primary use is as a bath treatment to control fungal diseases in fish, as well as in fish eggs prior to hatch. The commercially available 35% hydrogen peroxide solution is diluted before use in disinfection. The diluted solution to which fish and fish eggs are exposed contains 50 – 1000 mg/L hydrogen peroxide. Exposure durations at hatcheries range between 15 – 60 minutes/day, with the higher concentrations used in conjunction with the shortest exposure durations. Depending on the specific fungal infection, treatments can be repeated on multiple days, or on alternating days up to a total of three treatments/fish. Hydrogen peroxide is also believed to be effective against many bacterial and viral infections. It is not normally used to treat bacterial and viral infections in fish hatcheries, although it is beginning to be used to treat bacterial infestations of fish gills. The only two hatcheries in Washington currently reporting use of hydrogen peroxide are the Quilcene and Little White Salmon National Fish Hatcheries, both of which discharge to freshwater systems.

### Measures of Exposure:

Hydrogen peroxide is classified as a low regulatory priority aquaculture drug by the FDA (2006). Its use in hatcheries is generally for the control of external fungal infestations. It is also beginning to be used to treat bacterial infections of fish gills. Application is generally at a concentration between 50 – 1000 mg/L to fish and fish eggs. Both the Quilcene and Little White Salmon National Fish Hatcheries report using H<sub>2</sub>O<sub>2</sub> at a concentration of 1000 mg/L for 15 minutes/day. This use rate and concentration is in keeping with AFS (2011) recommendations for exposure concentration and duration to treat external fungal infections.

The Quilcene hatchery, but not the Little White Salmon hatchery, provided additional information to EPA regarding the daily volume of H<sub>2</sub>O<sub>2</sub> use and the number of days per year H<sub>2</sub>O<sub>2</sub> is used. This information, when combined with the measured range of water discharges from the Quilcene hatchery to receiving waters allowed us to calculate the concentration of hydrogen peroxide in hatchery discharges. These calculations are presented in the Expected Environmental Concentration (EEC) portion of this Measures of Exposure section.

In addition to its potential discharge from hatcheries, hydrogen peroxide is a naturally occurring chemical, produced by both biochemical and photochemical processes. It is found in freshwater at concentrations between 0.001 – 0.109 mg/L, and in marine waters at concentrations between 0.001 – 0.0136 mg/L (FDA 2006). Most organisms produce hydrogen peroxide under aerobic metabolism, which is then metabolically transformed into water and elemental oxygen (O<sub>2</sub>), primarily by the enzyme catalase. Hydrogen peroxide is freely soluble in water. Its estimated log octanol-water partition coefficient (log K<sub>ow</sub>) of -1.5, combined with the ability of organisms to rapidly metabolically transform hydrogen peroxide into water and oxygen are all indicative of a chemical with little ability to bioaccumulate.

The remainder of this measures of exposure assessment will evaluate two aspects that combined define the exposure of ESA listed species to hydrogen peroxide in the environment: its environmental fate once released into the environment, and its expected environmental concentration.

### ***Environmental Fate of Hydrogen Peroxide***

This section will describe the expected environmental fate of hydrogen peroxide.

Under non-sterile conditions in aerobic surface waters, the half-life of hydrogen peroxide is 1.1 – 5.3 hours (Breithaupt 2007). These are the conditions found in nearly all surface waters except for highly oligotrophic systems containing little in the way of organic matter and bacterial populations.

The two Washington hatcheries that currently use hydrogen peroxide (Quilcene, Little White Salmon) both treat fish for fungal and gill bacterial issues using an initial concentration of 1000 mg/L H<sub>2</sub>O<sub>2</sub>. Using the range of half-lives given in Breithaupt (2007), the concentration of H<sub>2</sub>O<sub>2</sub> remaining in water, assuming no dilution, after any given time period after the initial exposure can be estimated assuming first order degradation kinetics with the following two equations.

$$\lambda = \frac{\ln 2}{t_{1/2}}$$

Where:  $\lambda$  = Degradation rate (hour<sup>-1</sup>)

$t_{1/2}$  = Half-life of the chemical in the environment (hours), and

$$C_t = C_0 e^{-\lambda t}$$

Where:  $C_t$  = Chemical concentration in water at time  $t$  (mg/L)

$C_0$  = Initial chemical concentration in water (mg/L)

$\lambda$  = degradation rate (hour<sup>-1</sup>)

$t$  = Time elapsed after initial addition of chemical to water (hours)

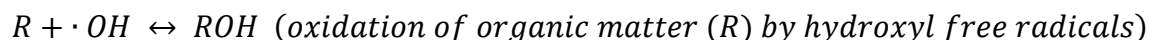
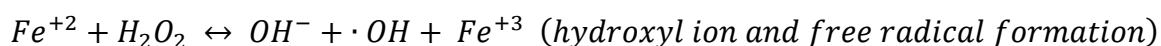
Table HP-??? shows estimated residual hydrogen peroxide concentrations in water after an initial addition of 1000 mg/L H<sub>2</sub>O<sub>2</sub>, using both the shortest (1.1 hours) and longest (5.3 hours) half-lives given by Breithaupt (2007) for H<sub>2</sub>O<sub>2</sub> in surface waters.

**Table HP-??? Hydrogen peroxide residual concentrations (mg/L) in surface water at different time periods after an initial concentration of 1000 mg/L, based on two different half-lives in water. Residual concentrations assume no dilution by additional water.**

Time after initial dose (hours)	Half-life = 1.1 hours	Half-life = 5.3 hours
0	1000	1000
1	533	877
2	284	770
3	151	675
4	80.4	593
6	22.8	456
12	0.52	208
18	0.012	95.0
24	0.00027	43.3
48	$7.31 \times 10^{-11}$	1.88
72	$1.98 \times 10^{-18}$	0.081

Under sterile conditions, and particularly sterile conditions in the absence of light, hydrogen peroxide solutions can remain stable for months, with only minimal reductions (approximately 2% reduction in H<sub>2</sub>O<sub>2</sub> / year) in the concentration of hydrogen peroxide. This is the reason commercially available solutions of hydrogen peroxide can be sold.

The primary reactions of hydrogen peroxide in surface water include the following:



Although ferrous iron (Fe<sup>+2</sup>) is shown in the above reaction, other metals, including manganese and several divalent cations can also serve as catalysts for the production of hydroxyl ions and hydroxyl free radicals (·OH). Most organic matter, including cell membranes and viral envelopes, is quickly oxidized by the hydroxyl free radicals released during the breakdown of H<sub>2</sub>O<sub>2</sub> in surface water. This oxidation of organic matter with hydroxyl free radicals is the primary mechanism of toxic action by which hydrogen peroxide serves as a disinfectant.

### ***Expected Environmental Concentration (EEC) of Hydrogen Peroxide***

The desired treatment concentration of hydrogen peroxide at the two hatcheries that currently report its use is 1000 mg/L. The Quilcene National Fish Hatchery has provided EPA with information regarding the volume of hydrogen peroxide used per day, the number of days per year H<sub>2</sub>O<sub>2</sub> is used, and a range of daily water discharges from the hatchery to receiving waters.

This information permits us to calculate the expected environmental concentration (EEC) of hydrogen peroxide in water at the point where the hatchery discharges into a receiving water (i.e. the end of pipe hydrogen peroxide concentration). This end of pipe concentration is used as a conservative estimate of the hydrogen peroxide concentration in receiving waters prior to any dilution of hatchery discharges by the receiving body of water. This EEC calculation also does not take into account the degradation of hydrogen peroxide described in the environmental fate portion of this Measures of Exposure section.

As described in the Problem Formulation section of the methodology used in this BE, the EEC is calculated as follows, based on procedures described in Schmidt et al. (2007).

$$EEC = \frac{C \times V}{F + E}$$

Where: EEC = Expected environmental concentration (mg/L or µg/L)

C = Treatment concentration of chemical in the hatchery (mg/L or µg/L)

V = Volume of chemical used (gallons/day)

F = Volume of water discharged from hatchery to receiving water (gallons/day)

E = Effluent pond volume (gallons)

For the purposes of calculating the hydrogen peroxide EEC, EPA has assumed that the effluent pond volume is zero. The Quilcene hatchery hydrogen peroxide use volume, concentration, and the hatchery low, average and maximum daily discharges to receiving water are presented in Table HP-???, along with the calculated EEC for each of the three hatchery discharge volumes.

**Table HP-???. Expected environmental concentration of hydrogen peroxide under low, average and high water volume daily discharges from the Quilcene National Fish Hatchery.**

Parameter	Value	EEC (µg/L)
Chemical use concentration, mg/L	1000	
Daily volume used, gallons	7.94	
Total volume used/year, gallons	286	
Days/year chemical used	36	
Low hatchery discharge, gallons/day	59,305	134
Average hatchery discharge, gallons/day	9,217,390	0.862
High hatchery discharge, gallons/day	31,966,747	0.249

EEC values in Table HP-??? do not take into account any degradation of hydrogen peroxide that occurs during the time between hatchery fish were exposed to H<sub>2</sub>O<sub>2</sub> and the time at which the exposure water was discharged into a receiving water. Because degradation of H<sub>2</sub>O<sub>2</sub> was not considered in the EEC calculations shown in Table HP-???, the EEC values presented are likely overestimates of the concentrations that would be discharged into surface waters. The EEC concentrations from Table HP-??? will be compared to the chronic NOEC estimates calculated in the Measures of Effect section. This comparison will take place in the Risk Characterization

section to estimate ecological risks to T&E species exposed to hydrogen peroxide discharges from hatcheries in Washington.

### Measures of Effect:

For fully aquatic species, the available toxicity data was identified from a search in EPA's ECOTOX database (<http://cfpub.epa.gov/ecotox/>).

A combined total of 321 toxicity records were identified from the above search. These results are presented in Appendix ??, Table ?? Of these records, only 10 exposed animals to hydrogen peroxide under flow through conditions: 9 records for *Daphnia magna* and one for rainbow trout. The one flow through exposure with rainbow trout (Powell and Perry 1997) only exposed the fish to hydrogen peroxide for one hour, not the 96 hour exposure called for by EPA in its data quality guidelines for a study to be useable in the derivation of EPA water quality criteria. Powell and Perry (1997) observed 100% mortality of rainbow trout in one hour when exposed to 1500 mg/L H<sub>2</sub>O<sub>2</sub>. Both the H<sub>2</sub>O<sub>2</sub> concentration and exposure duration in Powell and Perry (1997) are higher than the 15 minute exposure to 1000 mg/L H<sub>2</sub>O<sub>2</sub> used by hatcheries to treat fungal and bacterial infections.

The remaining available toxicity data for aquatic species was performed under static, static renewal or pulsed exposures. Taxa for which hydrogen peroxide toxicity data are available that does not meet EPA requirements for use in deriving water quality criteria are as follows:

- Freshwater algae: 13 species
- Freshwater macrophytes: 4 species
- Aquatic insects: 1 species
- Freshwater crustaceans: 4 species
- Freshwater zooplankton: 1 species
- Freshwater molluscs: 2 species
- Other freshwater invertebrate taxa (e.g. oligochaetes): 1 species
- Freshwater fish: 23 species
  
- Marine algae: 7 species
- Marine macrophytes: None
- Marine insects: None
- Marine crustaceans: 4 species
- Marine zooplankton: 4 species
- Marine molluscs: 4 species
- Other marine invertebrate taxa (e.g. polychaetes): 1 species
- Marine amphibians: None
- Marine fish: 7 species

Of the available toxicity data, some information on a T&E species under evaluation in this BE is for rainbow trout (steelhead), Chinook salmon and coho salmon. We have used the available 96 hour LC<sub>50</sub> data under static exposure conditions for rainbow trout, coho salmon and Chinook

salmon to estimate the toxicity of hydrogen peroxide to the remaining ESA listed salmonid species in Washington. We have used the methodologies described under the problem formulation section of this BE, specifically using ICE models. We have done this even though the rainbow trout, coho salmon and Chinook salmon 96 hour LC<sub>50</sub> studies were performed under static exposure conditions, not flow through conditions. Flow through conditions are particularly important for maintaining the desired exposure concentrations of chemicals such as hydrogen peroxide that degrade quickly under environmental conditions. Exposing organisms to chemicals that rapidly degrade under flow through conditions provides a greater likelihood that the exposure concentrations are as intended throughout the study, relative to the chemical degradation and subsequent reduction in exposure concentration that occurs over time during static or static renewal exposure conditions.

### ***Toxicity of Hydrogen Peroxide***

No toxicity studies with fish meeting EPA requirements for use in developing aquatic life criteria are available for hydrogen peroxide. Of the available data, the most useful in evaluating potential hydrogen peroxide toxicity to T&E species in receiving waters is a series of 96 hour LC<sub>50</sub> studies performed under static exposure conditions on two size classes of rainbow trout, coho salmon and Chinook salmon (Taylor and Glenn 2008). The Taylor and Glenn (2008) studies were performed at the Abernathy Fish Technology Center of the U.S. Fish and Wildlife Service (Longview, WA) using fish stocks native to Washington (rainbow trout, Chinook salmon) or Oregon (coho salmon).

Taylor and Glenn (2008) exposed two different size classes of fish to hydrogen peroxide. Their ‘small’ group of fish had a target body weight of 2 grams, while their ‘large’ group of fish had a target body weight of 10 grams. The 96 hour LC<sub>50</sub> values for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) are given in Table HP-???. Taylor and Glenn (2008) did not report confidence intervals around their LC<sub>50</sub> values.

**Table HP-??? Empirical 96 hour LC<sub>50</sub> values for three salmonid species as reported by Taylor and Glenn (2008).**

<b>Species</b>	<b>Size Class</b>	<b>LC<sub>50</sub> (mg/L)</b>
Rainbow trout	2 gram body weight	373
Rainbow trout	10 gram body weight	196
Chinook salmon	2 gram body weight	200
Chinook salmon	10 gram body weight	106
Coho salmon	2 gram body weight	231
Coho salmon	10 gram body weight	225

No empirical chronic toxicity data with hydrogen peroxide are available for rainbow trout, Chinook salmon or coho salmon. Therefore, the procedures given in the Problem Formulation are used to convert the empirical 96 hour LC<sub>50</sub> values in Table HP-??? to chronic NOEC concentrations. This calculation involves dividing the lower of the two available LC<sub>50</sub> values for each of the salmonid species in Table HP-??? by 2.27 to first derive a ‘LC<sub>LOW</sub>’ concentration.

The LC<sub>LOW</sub> is then divided by a default national acute-chronic ratio of 8.3 to calculate the chronic NOEC concentrations for rainbow trout, Chinook salmon and coho salmon. These calculated chronic NOEC values are presented in Table HP-2.

Output of all ICE models run with hydrogen peroxide for the three remaining T&E species (bull trout, chum salmon and sockeye salmon), genera or family with available data in ICE is shown in Table HP-1 (Catherine, this is another very wide spreadsheet, in the standalone file “Table HP-1 ICE models for bull trout chum sockeye salmon.xlsx”). Using the ICE model selection guidelines set forth in the problem formulation, models used to estimate chronic NOEC’s for salmonid species are highlighted in green and bolded in Table HP-1

A family level ICE model using the empirical rainbow trout LC<sub>50</sub> data was used as the starting point to derive chronic NOEC values for bull trout, chum salmon and sockeye salmon. The genus and family level ICE models using empirical coho salmon toxicity data as input could not be used to estimate toxicity to bull trout, chum and sockeye salmon, because the empirical toxicity data was outside of the useable range of the ICE regression between coho salmon and bull trout, chum and sockeye salmon. The empirical genus level Chinook salmon – bull trout also could not be used to estimate hydrogen peroxide toxicity to bull trout, chum and sockeye salmon, again because the empirical Chinook salmon toxicity data was outside of the useable range of the ICE regression. The family level ICE model between rainbow trout and bull trout, chum and sockeye salmon was selected from the remaining ICE models because of the large number of data pairs in the regression, and high  $r^2$  and cross-validation scores.

The remaining ICE models, with poorer predictive ability and which were not selected as the source of chronic NOEC’s are shown in red in Table HP-1. As described in the problem formulation, the lower 95% confidence interval of the predicted chronic NOEC, if available, is used as the chronic NOEC in this BE. All ICE models used for hydrogen peroxide generated lower 95% confidence intervals of the chronic NOEC, and are shown in this section.

No information is available in ICE for eulachon or any of the T&E rockfish species, genera or families in Washington (bocaccio, canary rockfish, yelloweye rockfish). Therefore, hydrogen peroxide effects on eulachon and the rockfish species cannot be quantitatively evaluated, and must be considered as a toxicological uncertainty in this BE. However, as neither the Quilcene nor Little White Salmon National Fish Hatcheries directly discharge to marine or estuarine waters, it is unlikely that hydrogen peroxide discharges from these two hatcheries would impact saltwater species such as eulachon or rockfish.

The final selected chronic NOEC values for bull trout, Chinook salmon, chum salmon, coho salmon, sockeye salmon and steelhead that were compared to the expected environmental concentration of hydrogen peroxide in receiving water environments are summarized in Table HP-2.

**Table HP-2. Chronic no effect concentrations (NOEC) for T&E salmonid species exposed to hydrogen peroxide.**

Species	Chronic NOEC (mg/L)	Source of chronic NOEC
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Bull trout	5.09	ICE model – family level
Chinook salmon	5.63	Empirical acute data (Taylor and Glenn 2008)
Chum salmon	5.09	ICE model – family level
Coho salmon	11.9	Empirical acute data (Taylor and Glenn 2008)
Sockeye salmon	5.09	ICE model – family level
Steelhead	10.4	Empirical acute data (Taylor and Glenn 2008)

## **Risk Characterization: Hydrogen Peroxide**

### ***Risks to T&E Fish Species from Hydrogen Peroxide***

Risks to T&E fish species for which toxic concentrations of hydrogen peroxide can be identified from the literature are calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the estimated environmental concentration is divided by the chronic NOEC for each T&E species to calculate a hazard quotient. Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this BE, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of a T&E species. Note that acceptable EEC values vary between species.

Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to T&E species. Note that since hydrogen peroxide is a naturally occurring chemical, whose sources include aerobic metabolism of fish, the EEC, and thus ecological risks from hydrogen peroxide cannot be set at zero.

Hazard quotients for the six T&E salmonid species for which toxicity data is available or could be estimated are presented in Table HP-???. Hazard quotients were calculated using the EEC generated from the lowest and highest daily discharge from the Quilcene hatchery, which results in the largest EEC range to which T&E species could be exposed.

**Table HP-??? Hazard quotients (HQ) for T&E species exposed to the range of estimated environmental concentrations (EEC) of hydrogen peroxide discharged by hatcheries.**

<b>Species</b>	<b>EEC range (mg/L)</b>	<b>Chronic NOEC (mg/L)</b>	<b>Hazard quotient range</b>
Bull trout	0.000249 – 0.134	5.09	0.000049 – 0.026
Chinook salmon	0.000249 – 0.134	5.63	0.000044 – 0.024
Chum salmon	0.000249 – 0.134	5.09	0.000049 – 0.026
Coho salmon	0.000249 – 0.134	11.9	0.000021 – 0.011
Sockeye salmon	0.000249 – 0.134	5.09	0.000049 – 0.026
Steelhead	0.000249 – 0.134	10.4	0.000024 – 0.013

All hazard quotients in Table HP-??? are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all hatchery discharge scenarios. Note that the EEC values do not take into account the rapid degradation of environmental concentrations of hydrogen peroxide. This is discussed more fully in the uncertainty analysis portion of risk



characterization, as it is likely the major uncertainty in this BE which overestimates potential ecological risks to T&E species.

### ***Risks to Potential Freshwater Prey of T&E Species from Hydrogen Peroxide***

Although not of a data quality useful for deriving EPA water quality criteria, a fairly substantial number of species have some hydrogen peroxide toxicity data available for them (Appendix ???, Table ???). The only toxicity study with hydrogen peroxide that appears to be of a suitable quality for use in EPA water quality criteria derivation is that of Meinertz et al. (2008), who performed a 21 day chronic flow through exposure of the cladoceran *Daphnia magna* to hydrogen peroxide. Endpoints evaluated by Meinertz et al. (2008) included survival, reproductive output, growth and population sex ratio. Growth was the most sensitive endpoint for *D. magna*, with growth reductions occurring within 21 days at H<sub>2</sub>O<sub>2</sub> concentrations  $\geq 0.32$  mg/L. *D. magna* reproductive output was unaffected at concentrations  $\leq 0.63$  mg/L, survival was unaffected at concentrations  $\leq 1.25$  mg/L, while sex ratio was unaffected at concentrations as high as 5.0 mg/L.

In addition to the Meinertz et al. (2008) study on the crustacean zooplankter *Daphnia magna*, empirical adverse effect toxicity data for hydrogen peroxide exists for 13 freshwater algal species, four aquatic macrophyte species, one aquatic insect, three crustaceans, two molluscs, one worm, one amphibian, and 23 freshwater fish species.

Despite the lack of studies of a quality that could be used to develop EPA water quality criteria, we have used the procedures outlined in the Problem Formulation (i.e. divide the acute toxicity value by 2.27, then dividing the LC<sub>LOW</sub> by a default acute-chronic ratio of 8.3 to obtain a chronic NOEC) to estimate chronic NOEC concentrations for prey of T&E fish species. Chronic NOEC concentrations of hydrogen peroxide to prey of T&E species is summarized in Table HP-5.

**Table HP-5. Toxicity of Hydrogen Peroxide to Freshwater Prey of T&E Listed Species**

<b>Organism Type</b>	<b>Chronic NOEC range (mg/L)</b>
Algae	0.086 – 55.7
Aquatic macrophytes	1.8 – 12.6
Aquatic invertebrates	0.20 – 53.1
Aquatic insects	20.5
Crustaceans	0.20 – 53.1
Zooplankton	0.32 – 1.25
Molluscs	0.53 – 0.83
Others (e.g. oligochaetes, etc.)	5.31
Amphibians	0.97
Fish	0.53 - 164

The most sensitive freshwater species to hydrogen peroxide appears to be the cyanobacterium (blue-green alga) *Microcystis pulverea*, with a three day EC<sub>50</sub> for reduction in population abundance of 0.71 mg/L under static exposure conditions (Drabkova et al. 2007). For algae, a three day exposure is considered a chronic exposure period, as multiple algal generations are

produced during a three day period. Conversion of this empirical EC<sub>50</sub> to a chronic NOEC yielded a value of 0.086 mg/L, the only chronic NOEC for a prey species lower than the highest calculated EEC of 0.134 mg/L. The *Microcystis* chronic NOEC is higher than both the average and maximum hatchery discharge EECs.

Fish species appear to have the widest range of sensitivity to hydrogen peroxide among the taxa for which empirical toxicity information is available. The most sensitive freshwater fish appears to be the northern pikeminnow, with a calculated chronic NOEC of 0.53 mg/L. The most tolerant fish species is sea lamprey exposed in freshwater, with a chronic NOEC of 164 mg/L. The chronic NOEC values for most fish species falls between 1 – 15 mg/L, with salmonids as a group among the more tolerant species of hydrogen peroxide exposures (salmonid chronic NOECs between 5.63 and 26.5 mg/L).

As all other prey species chronic NOECs are higher than the highest EEC for hydrogen peroxide, we conclude that hydrogen peroxide is not likely to adversely affect prey species of T&E fish species in Washington.

### ***Uncertainty Analysis of Hydrogen Peroxide Risk Characterization***

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this hydrogen peroxide evaluation. By far the largest uncertainty in this evaluation is the complete absence of toxicity data in the literature that would permit a quantitative evaluation of risks to T&E rockfish species from hydrogen peroxide use at fish hatcheries. This type of uncertainty is a true unknown in this BE. However, as the only two Washington hatcheries currently using hydrogen peroxide both discharge to freshwater streams, not marine or estuarine systems, eulachon and rockfish species are not currently exposed to any hydrogen peroxide releases from Washington hatcheries.

Variation of expected environmental concentrations in hatchery discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of hydrogen peroxide occurs only during a small portion of a year. This use pattern means that during much of the year, hydrogen peroxide is not released from a hatchery. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC<sub>50</sub>, EC<sub>50</sub>, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests.

The rapid environmental degradation rates of hydrogen peroxide in aquatic systems also introduce variation in exposure concentrations and EECs over time. Variation in hydrogen peroxide concentrations due to its environmental degradation is a unidirectional process, with the environmental concentration constantly declining. Without consideration of the degradation rate of H<sub>2</sub>O<sub>2</sub> in surface water, the EEC values used to describe exposure of T&E species to H<sub>2</sub>O<sub>2</sub> overestimate the concentrations T&E species are actually exposed to in the environment. Not attempting to estimate the effect on hydrogen peroxide EECs of dilution of hatchery discharges by receiving waters also serves to overestimate the actual EEC to which T&E species are exposed. Although we have estimated EECs and degradation rates separately in this BE, given

the already low hazard quotients calculated from our EECs, we have chosen not to modify our EECs by inclusion of a degradation rate term.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC<sub>50</sub> value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this BE for species where no empirical toxicity data exists for a chemical-species pair.

### **Effect Determinations of Hydrogen Peroxide on T&E Species**

Based on all chronic NOEC concentrations for six T&E salmonid species being substantially higher than the estimated environmental concentrations of hydrogen peroxide released from hatcheries, EPA has made the following effect determinations for hydrogen peroxide:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being substantially lower than the chronic NOECs for the above six species.

Based on the lack of current discharges from any Washington hatchery directly into estuarine or marine waters, the following species are not exposed to hydrogen peroxide releases from Washington hatcheries. Therefore, a no effect determination from hydrogen peroxide released by hatcheries is warranted for the following species.

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

These no effect determinations would need to be revisited if hatcheries which discharge directly into estuarine or marine systems would begin to use hydrogen peroxide in their operations at some future date.

### **Literature Cited**

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